Microscopic Studies on the Gonad of the Ascidian, Cynthia roretzi var. Drasche

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Microscopic Studies on the Gonad of the Ascidian, Cynthia roretzi var. Drasche.

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Summary

The systematic histological investigation has been made of the reproductive cycle in the ascidian, *Cynthia roretzi*. In general, it takes 6–8 months for gonad development, 1–2 months for spawning, and 2–4 months for recovery, but these periods are by no means clear-cut. The following division, based on histological observation, was used to determine seasonal gonad development. Namely, immature stage, developing stage, spawning stage and recovery stage.

In Mohne Inlet, the spawning usually occures from late December to middle January when the sea water temperature falls to 10°C. As a rule, an individual ascidian does not discharge all of its sex cells at one time. Usually the spawning continues for many days or even 2 weeks.

Full-grown oocytes are surrounded by five layers or envelopes. Both inner follicle cells and test cells appear to engage in active synthesis and secretion during the growth of the oocyte. The test cells are PAS-positive and react metachromatically with toluidine blue, suggesting that it may contain polysaccharide. The test cells appear to degenerate in the older oocytes. The outer follicle cells remain thin throughout the oogenesis and do not appear active in synthesis or secretion.

From November to December, the spermatogenesis proceeds at a very rapid pace. It is characteristic that spermatogenesis occurs rapidly in a short period. The sperm-head is stained a dense colour with basic dyes, and it is rod-shaped with "nebenkern" on the both sides of head.

Although numerous studies have been made in recent years on the gonad formation in various marine invertebrates, particularly in many bivalves, only a few papers have dealt with ascidians. The egg-follicle formation in *Cynthia roretzi* has been observed by Hirai (1939). He has observed that full-grown oocytes are surrounded by five layers of envelopes, namely basement membrane, outer follicle cell, inner follicle cell, chorion and test cell. However, no systematic

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investigation has been made of the reproductive cycle histologically, especially on the spermatogenesis.

In order to give a contribution to the comparative gametogenesis in the reproductive cycle of the ascidian, an economicaly important species, *Cynthia roretzi*, was studied in detail in the present paper.

Methods

Adult ascidians, *Cynthia roretzi*, were collected once every month from Mohne Inlet, Miyagi Prefecture, during the period from July of 1965 to June of 1966. For histological and cytochemical observations, ovaries and testes were excised in 10% formalin, Helly's and Carnoy's fixatives. After fixation, the tissues were dehydrated in a graded series of ethanol and embedded in paraffin. Sections were cut 6 μ in thickness and stained with hematoxylin-eosin, Mallory azan, toluidine blue, periodic acid-Schiff (PAS), Feulgen and Unna Pappenheim. Also, epoxy resin sections, 1 to 2 μ in thickness, were stained with toluidine blue for minute observation.

Results of Observations

Macroscopic observations

The reproductive organ, which is hermaphrodite and protogynous, is located on both the left and right sides of the body-wall in close contact with the stomach and the intestine. The ovaries on both sides are usually arranged in 7 to 10 rows, and their colour changes from yellow to dark brown as the maturation proceeds. The testis is made up of thin, white, branching tubules, which overlie a part of the ovary and often also a part of the intestinal wall. The oviduct and the vas deferens open into the anterior end of cloaca respectively.

Microscopic observations

The gametogenesis proceeds at a variable rate depending on the environment, such as sea water temperature. The seasonal variation of sea water temperature in Mohne Inlet is shown Fig. 1. The ascidian spawns naturally when the sea water temperature falls to 10°C in late December or in early January. Generally, it takes 6–8 months for gonad development, 2 months for spawning, and 2–4 months for recovery. For convenience, the cycle of gametogenesis was divided into the following four stages based on histological observations. They are, the immature stage (Fig. 2), the developing stage (Fig. 3), the spawning stage (Fig. 4) and the recovery stage.

Stages of the gonad development

1. *Immature stage*

Contrary to the case of the seminiferous tubule, the ovary is recognized

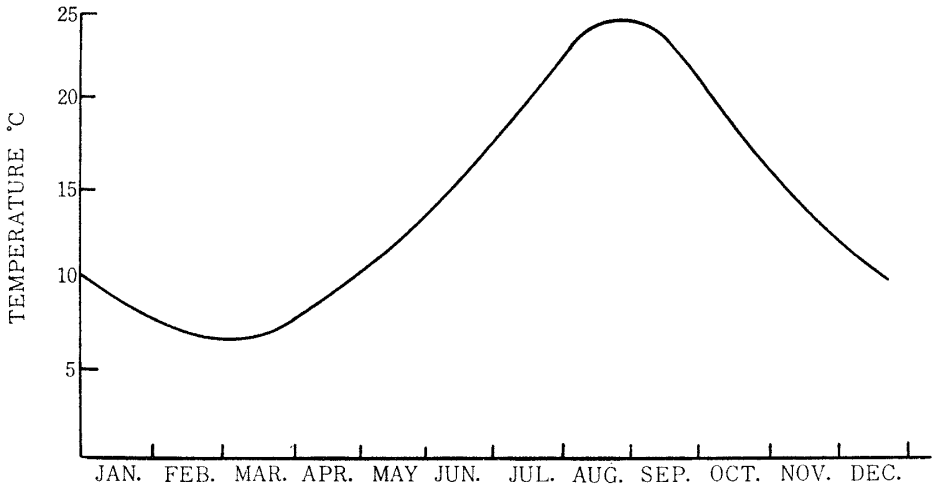

FIG. 1. Seasonal variation of sea water temperature in Mohne Inlet.

throughout the year as an organ containing egg cells in it. From March to June the ovaries are in a dormant state. The female sexuality is distinct because it contains a large number of oogonia and young oocytes. The cells at this stage are easily recognized by their large clear nucleus and basophilic cytoplasm.

After discharging the spermatozoa, in winter, the testis disappears and is not recognized until July (Fig. 2). In August, a few seminiferous tubules begin to appear and they are scattered in the form of small tubular vesicle throughout the connective tissue in the area between the ovarian rows (Fig. 10). The spermatogonia, occasionally with very few primary spermatocytes, form only a single row along the seminiferous tubule wall. The cells are often seen filling the seminiferous tubules completely. Spermatogonium is 6–8 μ in diameter and PAS-positive, and it contains a nucleus with a nucleolus.

2. *Developing stage*

Early in August, when the sea water temperature exceeds 20°C, the ovary is usually a yellowish colour. Young oocytes are surrounded by the inner and outer follicle cells which are scattered around the cells, and the cytoplasm contains a comparatively small number of yolk granules. As a rule, the cytoplasm of young oocytes show a conspicuous basophil (Fig. 6).

In late October, when the sea water temperature falls to 17°C, the oocytes start to grow gradually. Young oocytes become larger and increase in number. From early November to early December, the ovary turns yellowish borwn in colour. The test cells begin to migrate into the cytoplasm of the oocyte (Fig. 6). The cytoplasm of the test cell is completely separated from the oocyte cytoplasm by a plasma membrane. PAS reactive inclusions are observed both in the inner follicle cells and in the test cells. Staining with toluidine blue also shows a metachromatic mass in the test cells.

With further growth, the oocyte is completely surrounded by two layers of

follicular cells, and a thin, homogenous layer, the chorion or the vitelline membrane, which was formed between the inner follicular epithelium and the oocyte cytoplasm (Figs. 7, 8, 9). Throughout the oogenesis, the outer follicular envelope remains extremely thin. The oocyte is round with a diameter of 250 μ. The size of the nucleus and the nucleolus increases as the oocyte grows. Nucleolus and nucleus, with diameters of approximately 12 μ and 80 μ respectively, are observed in the full-grown oocyte. The staining reaction of the oocyte cytoplasm varies from intense basophil in the young oocytes, to intense acidphil in the larger oocytes containing yolk granules. The PAS reaction of the cytoplasm in the young oocytes is not intense, while in larger oocytes it is intensely positive. The chorion is also PAS-positive.

Macroscopically, the testis appears as white thready bodies, lying on the portion of the inter-ovarian connective tissue in late October or early November (Fig. 3). The seminiferous tubules increase in size and number. They contain comparatively large numbers of primary and secondary spermatocytes, and in some cases, even spermatids are formed. In later November, the seminiferous tubules gradually enlarge extending towards the inter-muscular connective tissue (Fig. 4). The spermatogenesis proceeds at a moderate pace, but spermatid and spermatozoa are observed in many individuals.

Early in December, spermatogenesis proceeds at a rapid pace. Towards the end of December, many individuals reach a fully ripened stage. The seminiferous tubules reach the region of the ovary and are separated from it by a thin layer composed of a few cells of connective tissue (Fig. 4). The central part of the seminiferous tubules is filled with a large number of matured spermatozoa (Fig. 11). The sperm-head or nucleus stains a homogenous, dense, dark colour with basic dyes. It is about 3 μ in length and rod-shaped. On both sides of the nucleus there are "nebenkern". The tail is very fine, about 30 μ in length, and stains with eosin (Fig. 13). A mass of them may be seen extruding into the vas deferens and getting ready for discharge.

3. *Spawning stage*

In the middle of December, when the sea water temperature falls to 12°C, the ovary is a dark brown colour. At this season, the ovaries of the individuals partially spawned can be easily recognized by the looseness of the ovary tissue.

In Mohne Inlet, the spawning usually occurs from later December to middle January when the sea water temperature falls to 10°C. As a rule, an individual ascidian does not discharge its entire content of sex cells at one time. Usually the spawning continues for many days or even for weeks.

After spawning, a looseness and a shrinking of the ovaries are observed (Figs. 5, 8). Only a few undischarged oocytes are left in the ovary. The ovarian rows rapidly diminish in thickness and the distance between each row gradually becomes large. The cytolysis and resorption of the undischarged full-grown oocytes

begin in later January. In the asicidan, the invation of phagocytic cells was not observed either inside or outside of the ovaries. But the cells of the connective tissue were seen penetrating into the intra- and inter-ovarian spaces.

As in the ovary, seminiferous tubules shrink after discharging a large quantities of spermatozoa. Spermatozoa undischarged are seen in the center of the lumina, but no spermatogonia or spermatocytes were observed to be left in the seminiferous tubules (Fig. 12).

4. *Recovery stage*

As the resorption of the undischarged oocytes and spermatozoa is completed, the ovaries gradually enter into the proliferation period and are soon to be filled with small young oocytes.

While in the testis, the seminiferous tubules become very small and are separated from each other by connective tissue, the seminiferous tubules remain in a dormant stage until the next spermatogenesis.

Discussion

The striking feature of the gametogenic cycle of the adult ascidian, *Cynthia roretzi*, is that they are protogynous. In the ovary, the growth of oocytes commences gradually in late spring. In early December, when the sea water temperature falls to 12°C, the oocytes are completely surrounded by a five layer envelope. At that time, the matured ovary turns dark brown in colour with many oocytes in a fully ripen stage.

On the other hand, the seminiferous tubules in the testis show almost no increase in size and number until later October. In November, the testis gradually begin to enlarge, and spermatogenesis proceeds at a moderate pace. Early in December, the spermatogenesis proceeds at a remarkably rapid pace and a very large number of spermatozoa are formed. The spawning occurs naturally when the sea water temperature falls to 10°C in the middle of December (3).

The morphological relationships of follicular epithelial cells and oocyte (4, 5, 6), at the stage approaching maturation, are as follows. The developing oocyte is surrounded by a follicular envelope separated from the oocyte by a thin vitelline membrane, commonly called the chorion. From the cytoplasmic morphology observed both in the inner follicular epithelial cells and in the oocyte it is suggested that the nutrient may possibly be transported through the vitelline membrane. It appears that the follicle cells synthesize nutritive material which is temporarily stored and then released into the matrix of the chorion. Then the nutrient may enter the oocyte cytoplasm through its plasma membrane. The outer layer of the follicle cells remains thin throughout oogenesis and does not appear to play any active role in synthesis or secretion. The chorion exists between the inner follicular epithelium and test cells, and it is of uniform thickness. The chorion

may be assumed, therefore, to be secreted by the inner follicular epithelium (1).

Of particular interest in ascidian oogenesis is the behaviour of the test cells in the oocyte (2), which have no counterpart in either vertebrates or invertebrates. In the case of *Styela rustica* and *Cynthia ocellata* with regard to the origin of the test cell, Sachxel (5) and Morgan (4) state that the test cells are formed by cell division which has taken place in the follicular epithelium, or the follicular cells have changed position. The test cells appear to be involved in active synthesis and secretion throughout the period of growth of the oocytes. The test cells maintain their individuality within the oocyte throughout its most active growing period, but may degenerate at the stage towards the end of oogenesis. The test cell may have a function somewhat like the follicular epithelium in helping to nourish the growing oocyte, but we have no explanation of their metabolism. A test cell is PAS-positive and reacts metachromatically with toluidine blue, suggesting that it may contain polysaccharide. Its origin and its functional significance remain to be solved

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Plate 1

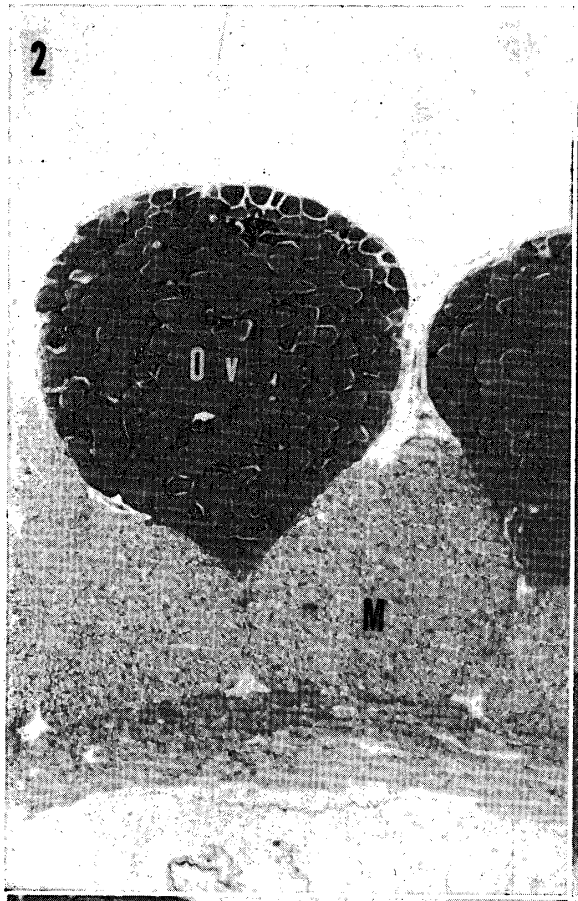
Explanation of Figures

FIG. 2. Micrograph of a cross section through a middle portion of the ovary (Ov). The predominant ovaries are seen in the early stages of spring oogenesis, but seminiferous tubules are not visible. Circular and longitudinal muscle fibers (M) constituting the body wall are well-developed. July × 30

FIG. 3. Micrograph of a cross section through a middle portion of the ovary (Ov) and the testis (T). In ovary, oocytes are showing rapid growth and some of them approaching ripeness, and in testis, proliferation of seminiferous tubules also become apparent. Circular and longitudinal muscle fibers (M) become considerably thinner than at earlier season. Late November × 30

FIG. 4. Micrograph of a cross section through a middle portion of the ovary (Ov) and the testis (T). Full-grown oocytes and spermatozoa are seen in the ovary and testis. Large numbers of circular and longitudinal muscle fibers (M) are showing shrinking. Late December × 30

FIG. 5. Micrograph of a cross section through a middle portion of the ovary (Ov) and the testis (T). The ovary and the testis of spawned ascidian are always easily recognizable. Spawning is characterized by the shrinkage of the ovary and the seminiferous tubules from which large number of the oocytes and the spermatozoa have already been discharged. Only oocytes and spermatozoa can be seen in the ovary and the seminiferous tubules respectively. The oviduct is seen in close to the ovary. Late January × 30


2
Ov
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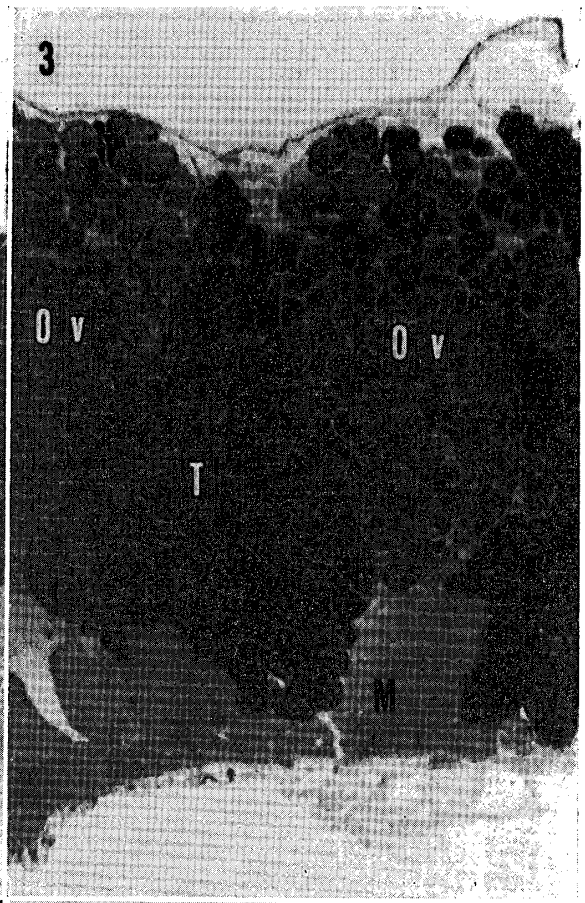
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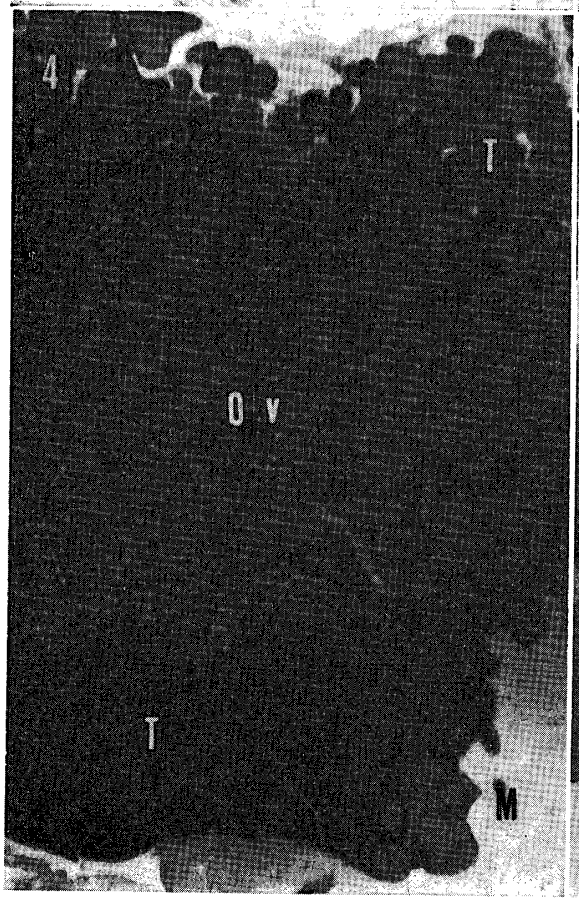
Ov
Ov
T
M


4
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Ov
T
M


5
Ov
Ov
T
M

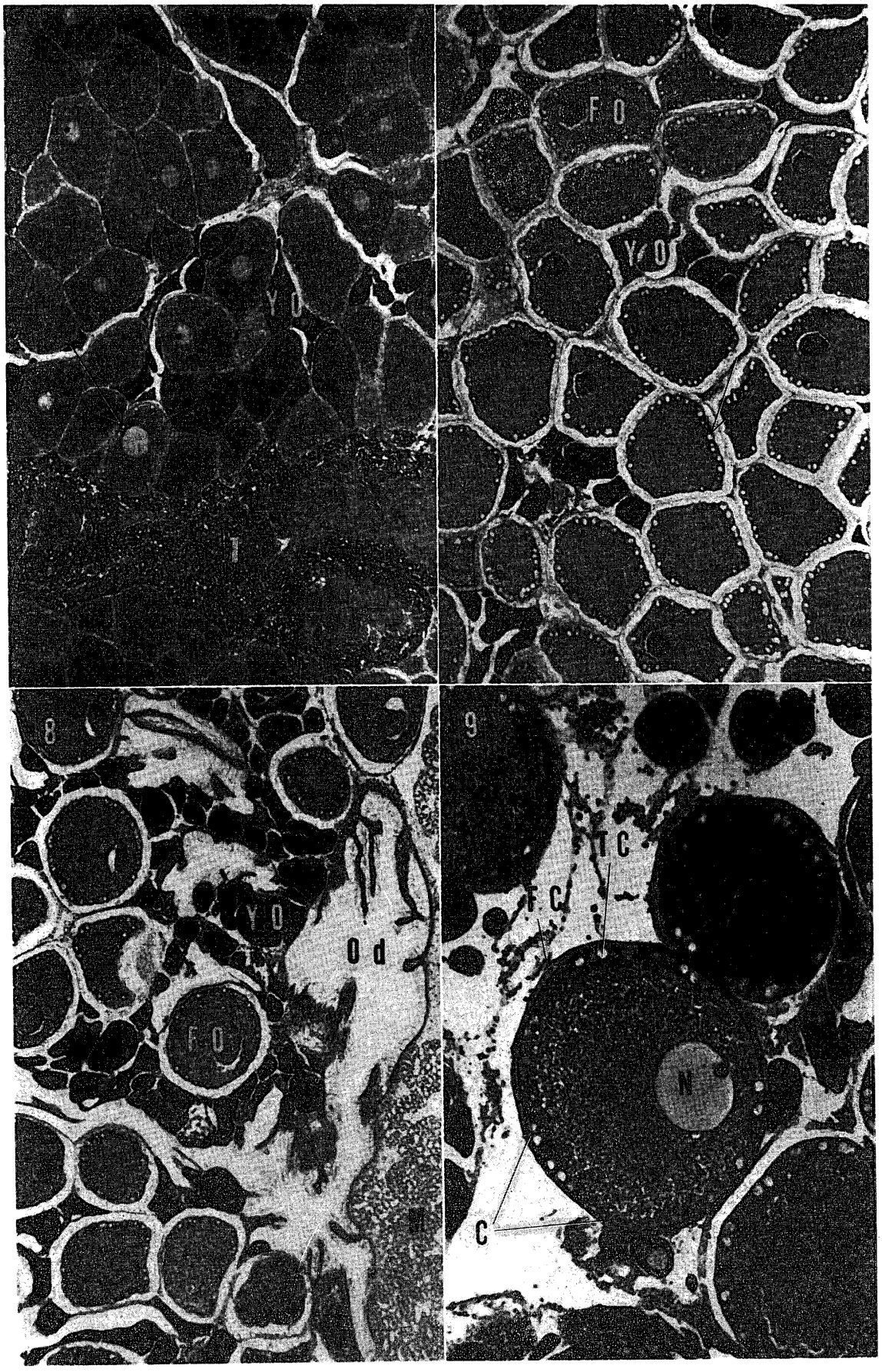

7
YO
FO
YO
8
YO
Od
FO
9
TC
FC
N
C

Plate 2

Explanation of Figures

FIG. 6. Micrograph showing a general view of the various stages of oocytes. Basophilic young oocytes (YO) are scattered along the ovarian walls. Oocytes are surrounded by the follicular envelope which is separated from the oocytes by a thin vitelline membrane, commonly called the chorion. In the most developed oocytes, the test cells are seen already (arrow). Seminiferous tubules (T) whose central part is occupied by large numbers of matured spermatozoa present between the ovaries. Late November × 60

FIG. 7. The full-grown oocytes (FO) are surrounded by basement membrane, outer follicle cells, inner follicle cells, chorion and test cells. The young oocytes are scattered among the full-grown oocytes. Spawning has not occurred yet. Late December × 60

FIG. 8. After spawning, the young oocytes (YO) are conspicuous among full-grown oocytes. The remaining undischarged full-grown oocytes (FO) are scattered in the ovaries. Finally, the full-grown oocytes disappear almost entirely, and young oocytes and oogonia remain until next season. By means of spawning the ovaries become loose and the oviduct (Od) is seen clearly. Circular and longitudinal muscle fibers (strata) (M) begin to show an increase in thickness. Late January × 60

FIG. 9. Micrograph to show relationship of follicle cell (FC), chorion (C) and test cell (TC). Nucleus of oocytes contain a clear nucleolus. × 150

Plate 3

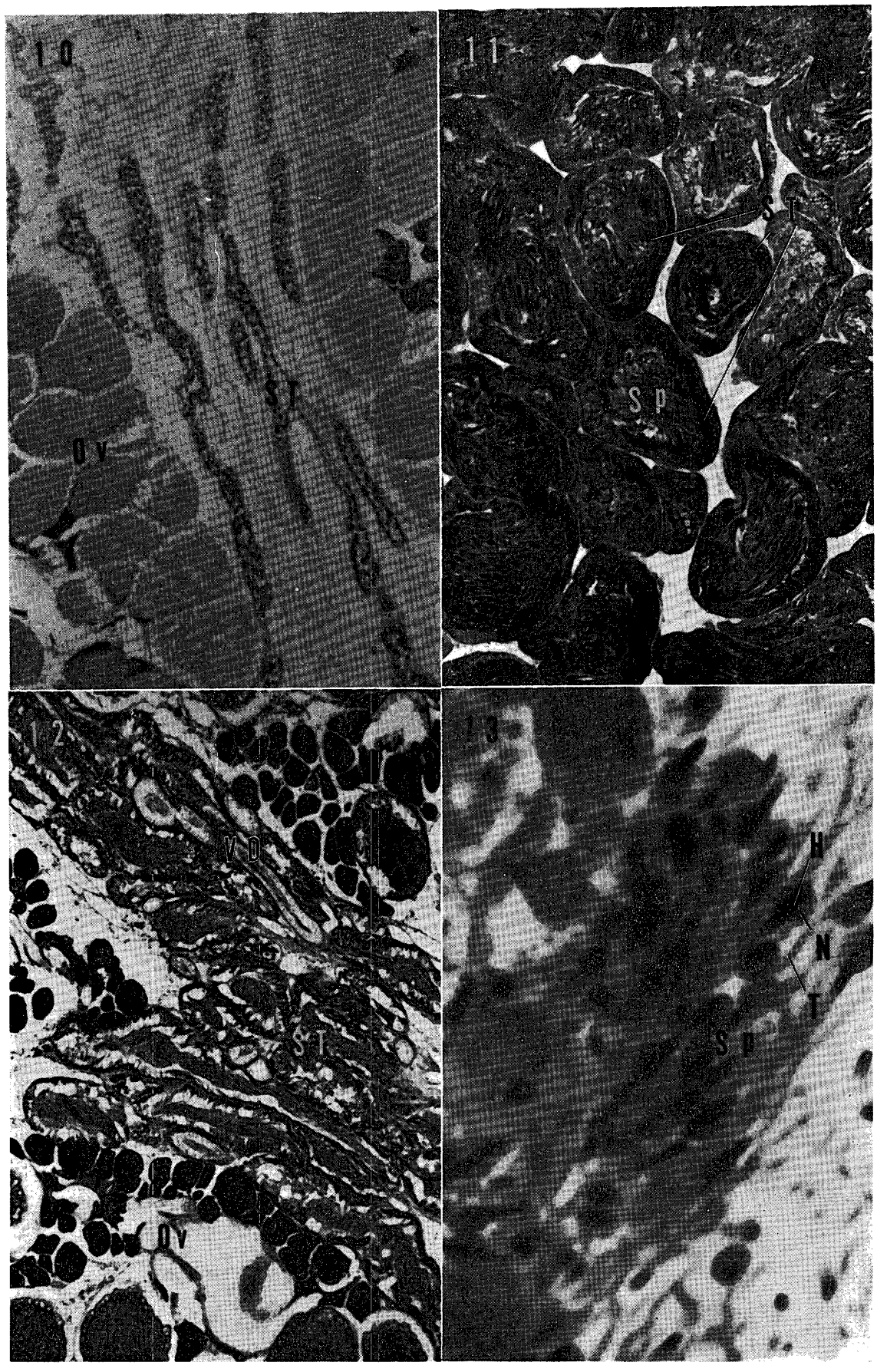
Explanation of Figures

FIG. 10. Micrograph of a cross section through a portion of inter-ovarian space. The seminiferous tubules (ST) are seen in the area confined inter-ovarian and inter-muscular connective tissues. They contain spermatogonia and very few primary spermatocytes. August × 60

FIG. 11. Spermatogenesis proceeds at a rapid pace, and in many samples spermatids and spermatozoa are already formed in large numbers. The central part of the seminiferous tubules (ST) are occupied by large numbers of matured spermatozoa (Sp). Late December × 60

FIG. 12. Micrograph of a cross section through a portion of inter-ovarian space. The seminiferous tubules (ST) are shrunk as a result of discharging large quantities of spermatozoa. Spermatozoa left are seen in the center of lumina. Sections of vas deferens (VD) are scattered among the seminiferous tubules. Late January × 60

FIG. 13. Micrograph of a high magnification of spermatozoa (Sp). Head (H), nebenkern (N) and tail (T) are clearly seen. × 1,500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ST
OV
11
ST
SP
12
VD
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13
H
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